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CREB Couples Neurotrophin Signals to Survival Messages

Minireview

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Nerve growth factor (NGF) is the prototypical member of the neurotrophin family of peptides, which includes brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5). Certain neurons survive a critical period during development, sustained by neurotrophins that are secreted by their innervation targets. If inadequate amounts of neurotrophin are available, these dependent neurons undergo programmed cell death. Since the discovery of NGF decades ago, many laboratories have sought to understand how neurotrophins promote the survival of neurons. These efforts have revealed specific pathways that convert neurotrophin signals arriving at the plasma membrane into adaptive intracellular biochemical responses. Parallel research into general mechanisms of cell survival has uncovered highly conserved molecular machinery that is responsible for executing programmed cell death. How upstream neurotrophin signaling impinges on the programmed cell death machinery to promote neuronal survival has been unclear. Two recent studies have shown that neurotrophins inhibit the cell death machinery, partly through the action of the cyclic AMP response element binding protein (CREB) family of transcription factors (Bonni et al., 1999; Riccio et al., 1999). This minireview discusses these findings and relates them to known functions of CREB and other transcription-dependent mechanisms of neuronal survival.

CREB the Transcription Factor

CREB belongs to a family of transcription factors that form homo- and heterodimers through a series of leucine residues and bind to DNA through adjacent basic amino acids (reviewed by Shaywitz and Greenberg, 1999). Diverse extracellular stimuli activate CREB through multiple signaling cascades that converge to phosphorylate a critical residue, Ser-133. The first neurotrophin-induced CREB kinase to be identified was the 90 kDa ribosomal S-6 kinase-2 (RSK-2), a downstream target of the Ras/ERK pathway (Figure 1). Subsequently, multiple neurotrophin-induced CREB kinases have been identified, including MAPK-activated protein kinase-2 (MAPKAP-2), Ca^{2+}- and calmodulin-dependent kinase IV (CaMKIV), and possibly protein kinase B (PKB/Akt) (Figure 2). The observation that diverse extracellular stimuli regulate CREB function has led to the idea that CREB and related family members play critical roles in developmental and adaptive responses that require stimulus-dependent transcription.

CREB and Development

Specific roles for CREB in development have been revealed through manipulations of CREB function in vivo and in vitro. Expression of a dominant-interfering CREB within certain pituitary neurons causes them to develop abnormally or fail to form altogether (Struthers et al., 1991), and mice lacking the CREB gene exhibit a defect in T cell development and die perinatally (Rudolph et al., 1998). Some of the developmental defects were attributed to reduced cell proliferation; however, recent experiments in granulosa cells distinguished a role for CREB in regulating cell survival, apart from possible roles in differentiation or proliferation (Somers et al., 1999). Thus, CREB might regulate development through differential effects on cell proliferation and survival, depending on the cell and its stage of differentiation.

A mechanism whereby CREB might regulate cell survival during lymphocyte development emerged from analysis of *bcl-2* transcription (Wilson et al., 1996). Bcl-2 belongs to a family of proteins that suppress (e.g., bcl-2) or induce (e.g., BAD) apoptosis. Certain stimuli that promote B cell survival trigger both CREB phosphorylation and *bcl-2* transcription. Promoter analysis of the 5′ regulatory region of the *bcl-2* gene revealed a consensus cyclic AMP response element (CRE) that binds CREB and related family members and whose mutation blocks stimulus-induced *bcl-2* transcription (Wilson et al., 1996). Pugazhenthi et al. (1999) extended these findings by showing that the growth factor, insulin-like growth factor-1 (IGF-1), induces *bcl-2* transcription in PC12 cells through CREB. CREB regulation of *bcl-2* transcription suggested a way that other extracellular signals, such as neurotrophins, might promote neuronal survival.

In vivo studies suggested that bcl-2 or a close family member might mediate neurotrophin-dependent survival. Disruption of *bcl-2* led to the postnatal death of

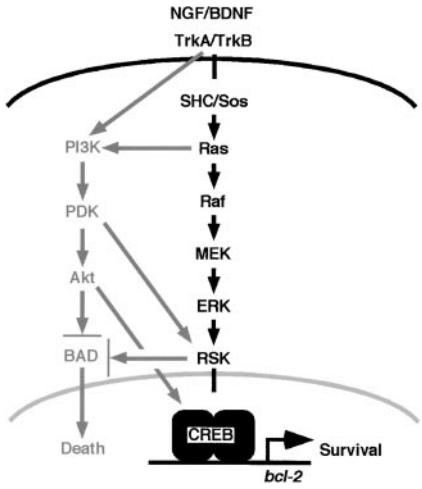

Figure 1. A Diagram of Signal Transduction Pathways that Mediate Neurotrophin-Induced Neuronal Survival

The CREB-dependent pathway (bold) can be triggered by NGF acting through its cognate receptor, TrkA, or by BDNF through TrkB. PI3-K, phosphatidylinositol 3-kinase; PDK, phosphoinositol (3,4,5) tris-phosphate-dependent kinase; Sos, son of sevenless; MEK, MAPK kinase.

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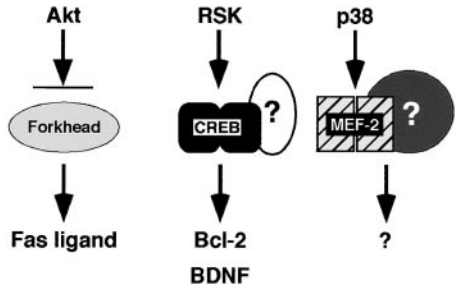

Figure 2. Multiple Transcription-Dependent Pathways Regulate Neuronal Survival

CREB and MEF-2 are believed to cooperate with other promoter-bound factors to regulate survival; however, the identities of the other factors are not known (indicated by question marks).

sympathetic and sensory neurons (Michaelidis et al., 1996), whereas overexpression of *bcl-2* protected these same neurons from NGF withdrawal (reviewed by Deshmukh and Johnson, 1997). The discoveries that CREB is a major mediator of neurotrophin-induced transcription (Ginty et al., 1994; Finkbeiner et al., 1997) and that it regulates *bcl-2* suggested that CREB might regulate genes that mediate neurotrophin-induced neuronal survival. Recent reports from the Ginty (Riccio et al., 1999) and Greenberg (Bonni et al., 1999) laboratories directly support this idea.

Neurotrophins Promote Neuronal Survival via CREB-Dependent Transcription

Riccio et al. (1999) explored a role for CREB by injecting and overexpressing different CREB mutants in sympathetic neurons and assessing their effects on NGF-induced survival. One mutant, CREBm1, binds to endogenous CREs but cannot mediate transcription because Ser-133 has been mutated to prevent its phosphorylation. Another mutant, A-CREB, dimerizes with specific endogenous CREB family members through their DNA binding sites, preventing them from binding to endogenous CREs and activating transcription. Injection of either CREBm1 or A-CREB blocked NGF-induced survival, consistent with a model that places CREB downstream of NGF pathways that promote the survival of early postnatal sympathetic neurons.

If NGF induces the expression of critical survival genes through CREB or related family members, it might be possible to directly express these genes and rescue neurons from death induced by NGF withdrawal. To force transcription of CREB target genes, a constitutively active mutant of CREB was constructed (CREB-VP16) by replacing its inducible activation domain with a constitutive activation domain from VP16. As predicted, a version of CREB-VP16 that could bind to CREs enabled neurons to survive NGF withdrawal. The observations suggest that CREB-VP16 binds to endogenous CREs and promotes the transcription of specific genes that mediate neuronal survival.

To identify candidate survival genes regulated by CREB, Riccio et al. (1999) screened NGF-stimulated cells by cDNA array analysis. Subsequent Northern and Western analyses confirmed that one gene, *bcl-2*, was induced by NGF in sympathetic neurons and PC12 cells and by BDNF in cortical neurons. Reporter gene analysis showed that the known CRE within the *bcl-2* promoter was critical for its NGF responsiveness. Subtle mutations in the *bcl-2* CRE or the coexpression of dominant-interfering CREB blocked NGF-induced *bcl-2* reporter gene transcription. Conversely, transfection with CREB-VP16 led to the overexpression of endogenous *bcl-2*, and *bcl-2* expression rescued sympathetic neurons from the pro-apoptotic effects of transfected A-CREB or CREBm1. Taken together, the findings suggest that NGF promotes sympathetic neuron survival partly through CREB-dependent transcription of *bcl-2*.

How important is CREB-dependent transcription for neurotrophin-induced survival of other types of neurons? Bonni et al. (1999) investigated how BDNF, signaling through the Ras/ERK pathway, promotes the survival of cerebellar granule cells. Programmed cell death of granule cells can be prevented by adding specific factors, such as IGF-1, BDNF, or depolarizing amounts of potassium chloride, to minimal medium. PI-3K and Akt/PKB signaling are critical for IGF-1-dependent survival (reviewed by Datta et al., 1999) and may play a role mediating depolarization-induced survival (Vaillant et al., 1999). In addition, depolarization promotes granule cell survival through a pathway that depends on p38 and the transcription factor, myocyte enhancing factor-2 (MEF-2) (Mao et al., 1999). Although BDNF was known to Akt/PKB and the Ras/ERK pathway, it had been unclear whether the Ras/ERK pathway promoted granule cell survival by mechanisms independent of its ability to activate the Akt/PKB pathway.

To investigate a specific role for the Ras/ERK pathway, Bonni et al. (1999) first showed that inhibition of the Ras/ERK pathway selectively attenuated BDNF- but not IGF-1- (or insulin-) regulated granule cell survival. Since inhibition of the Ras/ERK pathway occurred downstream of the point at which Ras activates PI3-K, the Ras/ERK pathway appeared to promote granule cell survival by mechanisms that were partly independent of Akt/PKB (Figure 1).

One downstream Ras/ERK effector, RSK-2, can phosphorylate and regulate a variety of cellular substrates, including CREB. In a convincing set of experiments, Bonni et al. (1999) showed that BDNF promotes granule cell survival through RSK-2 by directly inhibiting the pro-apoptotic *bcl-2* family member BAD and by transcription-dependent mechanisms involving CREB. Using mutants to assess the role of CREB in BDNF-induced granule cell survival, Bonni et al. (1999) obtained results in granule cells similar to those of Riccio et al. (1999) in sympathetic neurons. Overexpression of dominant-interfering CREB blocked BDNF-stimulated granule cell survival, whereas constitutively active CREB kept granule cells alive in the absence of BDNF. Bonni et al. (1999) also reported that BDNF induced the expression of a *bcl-2* reporter gene via CREB. The findings of Bonni et al. (1999) and Riccio et al. (1999) identify a new pathway by which neurotrophins promote the survival of peripheral and central nervous system neurons and establish CREB as a critical regulator of neurotrophin-dependent neuronal survival.

Remaining Questions and Future Directions

There are several interacting pathways for survival, death, *bcl-2* transcription, and CREB Ser-133 phosphorylation. Some of this richness is described below. For

example, although CREB-dependent activation of *bcl-2* transcription is an attractive mechanism to explain neurotrophin-induced survival, the contribution of this pathway to programmed cell death in vivo remains to be established. In vivo, the absence of NGF, its cognate receptor TrkA, or *bcl-2* causes overlapping populations of neurons to die. However, significant neurodegeneration in *bcl-2*$^{-/-}$ mice appears to occur after a time point (P10) when the majority of these neurons are lost in NGF$^{-/-}$ or TrkA$^{-/-}$ mice, and loss of cerebellar granule neurons in *bcl-2*$^{-/-}$ mice has not been reported (Michaelidis et al., 1996). Moreover, BDNF is capable of promoting the survival of motoneurons following axotomy, even in *bcl-2*$^{-/-}$ mice (Michaelidis et al., 1996). Thus, additional neurotrophin-dependent signal transduction pathways and CREB target genes may mediate neuronal survival. To test this possibility, it would be interesting to determine if CREB-VP16 can promote the survival of sympathetic neurons or cerebellar granule cells from *bcl-2*$^{-/-}$ mice.

Support for the existence of additional transcription-dependent survival pathways has appeared recently (Figure 2). Akt/PKB mediates neuronal survival partly through phosphorylation and inhibition of FKHRL1, a member of the Forkhead family of transcription factors (reviewed by Datta et al., 1999). FKHRL1 phosphorylation prevents it from translocating to the nucleus and upregulating the proapoptotic protein Fas ligand. Recently, the ability of Ca^{2+} influx to promote granule cell survival was shown to depend partly on the activation of MEF-2-dependent transcription (Mao et al., 1999). Taken together, these results establish a commonly held idea—that a major mechanism by which extracellular stimuli such as neurotrophins and neuronal activity regulate the survival of specific populations of developing neurons is through the activation or repression of gene transcription. Thus, a major focus for future research will be to identify the critical target genes that mediate survival and to discover how factors, such as CREB, regulate their transcription.

For some survival genes, CREB may need to interact with other promoter-bound factors to achieve physiologic patterns of gene expression. The *bcl-2* gene contains an upstream response element (URE) that is adjacent but upstream of the CRE (Wilson et al., 1996). Although an isolated URE cannot mediate *bcl-2* expression, the URE cooperates with the adjacent CRE to attain levels of *bcl-2* reporter gene expression that are double those achieved with the CRE alone (Wilson et al., 1996). In this regard, *bcl-2* is organized similar to BDNF, another CREB-regulated gene that mediates neuronal survival. Ca^{2+}-stimulated BDNF transcription depends on interactions within a BDNF promoter between CRE-bound factors and proteins that are bound to an adjacent URE (reviewed by Shieh and Ghosh, 1999). However, the identity of upstream factors that cooperate with CREB to regulate BDNF or *bcl-2* transcription and the significance of the URE/CRE organization remain unknown.

Response elements in addition to the URE and CRE may also mediate NGF-induced *bcl-2* transcription (Wilson et al., 1996). Downstream of the known CRE, *bcl-2* contains a negative regulatory element that binds the tumor suppressor p53. Kaplan and colleagues recently showed that NGF activation of the Ras/ERK pathway regulates the survival of sympathetic neurons partly by suppressing the p53-mediated cell death pathway (Mazzoni et al., 1999). Thus, NGF and other neurotrophins might control the expression of *bcl-2* positively through CREB and negatively through p53 suppression.

The discovery that CREB is a critical regulator of NGF-induced *bcl-2* transcription and neuronal survival raises the interesting possibility that CREB and related family members might play a general role in mediating stimulus-dependent cell survival. The critical Ser-133 on CREB that is phosphorylated by RSK-2 is also a target of many other signal transduction pathways besides the Ras/ERK pathway. Several of these pathways, such as protein kinase A (PKA), PKC, and CaMKIV, are activated by extracellular stimuli that promote the survival of some neuronal and nonneuronal cell populations. The observation that mice lacking CREB mostly survive until birth and apparently exhibit limited anatomical and physiological defects could be construed as evidence against a broad role for CREB in survival (Rudolph et al., 1998). However, other CREB family members probably substitute for the absence of CREB in these mice (Rudolph et al., 1998).

CREB has already been implicated in mechanisms of activity-dependent synaptic plasticity (reviewed by Silva et al., 1998). Now, the reports from the Ginty and Greenberg laboratories show that CREB also regulates neuronal survival. How does the same transcription factor mediate apparently distinct biological functions? CREB is a single component of a complex of promoter-bound enhancing factors. Whether a particular gene is transcribed in response to a signal is determined by the factors bound to the gene's promoter and the activity of the pathways that regulate the complex. Therefore, specificity may reflect the activity of parallel pathways which regulate factors that cooperate with CREB. However, it is tempting to speculate that the signal transduction pathways and programs of gene expression involved in activity-dependent survival and activity-dependent plasticity may largely overlap. For example, neurotrophic factors, extracellular Ca^{2+} influx, Ras/ERK pathway activation, and now CREB-dependent transcription have been assigned dual functions, regulating both neuronal survival and synaptic plasticity (reviewed by Curtis and Finkbeiner, 1999). A molecular connection between pathways that regulate survival and plasticity could lend support to the idea that some neurodegenerative disorders may ultimately prove to be a failure of plasticity (Mesulam, 1999).

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